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GAMMA-IRRADIATED VENEZUELAN EQUINE ENCEPHALITIS VACCINES

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DEPARTMENT OF THE ARMY
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and

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BIOLOGICAL SCIENCES LABORATORIES

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

The efficacy of formalin-inactivated Venezuelan equine encephalitis (VEE) vaccine has been reported to be low for man. Although a live VEE vaccine has been shown to be highly effective for the protection of laboratory workers, local and systemic reactions have occurred in approximately 20% of inoculated individuals. Therefore, studies were initiated in an attempt to produce an inactivated vaccine of high potency and low reactogenicity. Inactivated VEE vaccines were prepared by exposing virus suspensions to 8×10^6 or 10×10^6 r of gamma radiation. Irradiated VEE vaccines prepared from virus suspensions produced in Maitland-type chick embryo (MICE) cell cultures and in monolayer cultures of human diploid strain WI-38 cells were highly immunogenic for mice and guinea pigs. Guinea pigs vaccinated with a series of three inoculations of vaccine (MICE) survived challenge with at least $10^{8.4}$ $MICLD_{50}$ of VEE virus. Irradiated vaccines induced high levels of serum-neutralising and hemagglutinin-inhibiting antibodies in guinea pigs and rabbits. These findings suggest that ionizing radiation may be effective in the preparation of an inactivated VEE vaccine.

I. INTRODUCTION*

The preparation of inactivated vaccines by exposing suspensions of microorganisms to ionizing radiation has been reported for bacteria[1] and viruses.[2] The X rays and gamma rays that are used for inactivation are short-wavelength electromagnetic radiations that have high penetrating ability and have the desired characteristic of not imparting radioactivity to the exposed material. Studies with bacterial and animal viruses have shown that infectivity of viruses may be selectively destroyed by radiation while leaving antigenicity intact.[3-5]

Unlike chemical treatment, which continues to act on the antigenic material in vaccines until the chemical is either removed or neutralized, the effect of radiation ceases upon completion of the exposure process.

Two types of Venezuelan equine encephalitis (VEE) virus vaccines have been produced and tested in man. A 0.4% formalinized chick embryo vaccine prepared by Randall[6] has been used to vaccinate laboratory workers. Although this vaccine passed safety tests in guinea pigs, 14 human infections occurred in inoculated individuals.[7] Repeated attempts by investigators in three laboratories to isolate virus from the various lots of vaccine under suspicion, by inoculation of the vaccine into a large number of mice, guinea pigs, monkeys, rabbits, "wet" chicks, and embryonated eggs, were unsuccessful. The authors suggested that man may be a more sensitive test organism for the detection of live VEE virus. A safe formalin-inactivated vaccine was then prepared by Smith, Katz, and Wagner[8] by treating the virus suspension with 4% formalin. Seventy-five per cent of the individuals vaccinated with this vaccine developed a significant rise in hemagglutination-inhibition (HI) and serum-neutralization (SN) antibody titers when inoculated with an attenuated VEE virus strain, indicating a vaccine effectiveness of only 25%.[9]

An effective live VEE vaccine has been prepared in fetal guinea pig heart cell monolayers.[10] This vaccine has been reported to protect vaccinees against challenge with virulent VEE virus[9] and currently is being used to immunize laboratory workers at Fort Detrick. A single inoculation of 5×10^3 guinea pig intraperitoneal median immunizing doses stimulated the production of significant amounts of hemagglutination-inhibiting antibodies in approximately 96% of the vaccinees.** During the 6 years the vaccine has been in use, no clinical cases have been identified in those individuals who demonstrated a serological response to the vaccine. However, among vaccinated employees working in VEE-risk environments, a routine serological surveillance program has identified cases of VEE infection in three individuals who did not demonstrate an

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** R.W. McKinney, personal communication, 1969.

adequate response to vaccination with the live vaccine. These cases have been associated with presumed or documented accidental exposure to VEE virus and, in retrospect, had mild clinical symptoms compatible with VEE infection. Reactogenicity associated with the viable vaccine has been recorded in approximately 20% of the vaccinees; symptoms reported are similar to those characterizing grippal or influenza-like syndromes.*

In view of the reactogenicity associated with the use of the live vaccine and the poor potency of formalin-inactivated vaccines, the present study was undertaken in an attempt to produce an inactivated VEE vaccine with potency approaching that of the viable vaccine but associated with a lesser reaction potential in humans. This report presents the preliminary data in animals oriented toward the development of such a vaccine.

II. MATERIALS AND METHODS

A. VIRUS

The Trinidad donkey brain strain of VEE virus[11] was obtained at this laboratory as a 10% chick embryo (12th passage) suspension in beef heart infusion broth (BHIB). Seed virus was prepared from the 13th and 14th egg passages by inoculating $10^{5.3}$ mouse intracerebral LD_{50} of the virus into 10-day embryonated eggs via the allantoic route. The infected embryos were harvested after 24 hours' incubation at 35 C, and a 15% embryo suspension was prepared in BHIB containing 400 units of penicillin and 400 µg streptomycin per ml. Virus seed was stored in sealed ampules at -70 C in a mechanical freezer.

B. IRRADIATION

Virus suspensions were irradiated with a 50,000-curie cobalt^{60} source at the National Bureau of Standards, as described in a previous communication.[18] Irradiation doses are expressed as total doses in roentgens (r).

C. TITRATION OF VIRUS SUSPENSIONS

Infectivity titers of virus were determined by intracerebral (IC) and intraperitoneal (IP) inoculation of 0.03 ml and 0.2 to 0.25 ml, respectively, in 10- to 14-g Swiss mice, and by plaque assay in mouse fibroblast strain L cells. The 50% lethal dose end points were calculated according to the method of Reed and Muench.[19]

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* P.J. Kadull, personal communication, 1969.

D. PREPARATION OF VACCINES

Tissue culture virus suspensions were prepared in chick fibroblast monolayers (CF), in Maitland-type chick embryo suspensions (MICE) as described previously, and in monolayers of WI-38 cells.* Virus suspensions were clarified by centrifuging at 700 x g in an I.E.C. PR-2 refrigerated centrifuge.** Two lots of vaccine were partially purified by differential centrifugation. The virus suspension was centrifuged in a Spinco Model L ultracentrifuge at 8,720 x g for 15 minutes to remove debris, the supernatant fluid was then centrifuged at 54,500 x g for 60 minutes. and the virus pellet was resuspended in maintenance medium. Virus suspensions were distributed into serum bottles that were then sealed with rubber caps, frozen at -70 C, and inactivated by irradiation in the frozen state. Vaccines were kept at -70 C in a mechanical freezer until assayed.

E. SAFETY TESTS

Irradiated vaccines were tested for residual live virus by challenge of five litters of suckling mice and by the antigenic capacity of a single inoculation in the guinea pig as described previously.[6] No residual live virus was detected in virus suspensions exposed to 8×10^6 r or greater. Sterility of irradiated vaccines was determined by inoculation of liquid thioglycollate medium (Difco), Sabouraud dextrose agar, and nutrient agar.

F. VACCINE ASSAY

The 50% effective dose (ED_{50}) of the vaccines was determined according to the method of Cole and McKinney[14] by challenging animals IP with approximately $10^{5.4}$ $MICLD_{50}$ of the homologous strain of VEE virus 21 days after administration of the last dose of vaccine. The ED_{50} is defined as that quantity of undiluted vaccine given in each dose of the series that protects 50% of the animals from death after challenge. Irradiated vaccines were thawed in a 37 C water bath just prior to inoculation of 10- to 40-g male mice (Swiss), or 250- to 350-g Hartley strain guinea pigs of both sexes. The animals received one, two, or three IP inoculations of vaccine spaced at intervals of 1 week. Inoculations were administered in amounts of 0.2 or 0.25 ml and were scheduled to allow for completion of the various series at the same time. Thus, animals immunized with one inoculation received their inoculation at the same time that animals immunized with three inoculations were administered their final inoculation.

Antibody levels were determined by SN[7] and HI tests with pre- and postvaccination serum. HI tests were performed with an irradiated VEE hemagglutinin.[18] SN titers are expressed as $\log_{10}$; HI titers as the reciprocal.

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* Reitman, M. 1970. Appl. Microbiol. 19:(in press).

** International Equipment Co., 300 Second Ave., Needham Heights, Mass. 02194.

III. RESULTS

A. POTENCIES OF IRRADIATED VACCINES PRODUCED IN AVIAN AND MAMMALIAN TISSUES

Preliminary assays of the potencies of irradiated preparations were performed in mice. Table 1 lists the ED_{50} values obtained with experimental vaccines prepared from virus suspensions with preirradiation titers ranging from 8 to 10 logs $MICLD_{50}$/ml of infective virus.

TABLE 1. POTENCIES OF IRRADIATED VENEZUELAN EQUINE ENCEPHALITIS VACCINES FOR MICE

Vaccine	Cell Culture Preparation	Preirradiation Titer, $\log_{10}$ $MICLD_{50}$/ml	Radiation dose, r x 10^6	ED_{50} ml No. of Inoculations[a] 2	3
MR5	CF	8.1	8	0.062	0.0062
MR15	MTCE	10.0	10	0.01	0.0051
	MTCE[b]	8.6	10	>0.16	0.017
MR17	MTCE[b]	8.3	10	0.01	0.0051
MR27	MTCE	9.9	8	0.0036	
			10	0.015	
			16	0.015	

a. Mice were inoculated with 0.25-ml amounts of vaccine at 7-day intervals.
b. Partially purified.

Table 2 shows that the vaccines tested in guinea pigs had satisfactory potency values when administered in a series of two or three inoculations. In addition, smaller ED_{50} values were obtained in the guinea pig than in the mouse. The data indicate that irradiation is a satisfactory tool for the preparation of potent VEE virus vaccines.

B. EFFECT OF IRRADIATION ON ANTIGENICITY

Table 3 lists the ED_{50} values for vaccines that were exposed to various doses of irradiation. A dosage of 10 x 10^6 r caused approximately a 100-fold decrease in the potency of the WI-38 vaccine, but had no deleterious effect on the MTCE vaccine. Exposure of the latter to an irradiation dose of 16 x 10^6 r caused approximately a 10-fold decrease in potency; no potency was demonstrable after a dosage of 32 x 10^6 r.

TABLE 2. POTENCIES OF IRRADIATED[a] VENEZUELAN EQUINE ENCEPHALITIS VACCINES FOR GUINEA PIGS

Vaccine	Cell Culture Preparation	Preirradiation Titer, $\log_{10}$ $MICLD_{50}$/ml	ED_{50}, ml No. of Inoculations 1	2	3
MR25	MTCE	9.0	0.16[b]	0.0069	
MR27	MTCE	9.9	>0.04	0.0016	0.00059
50/2	WI-38	9.1		0.0046	0.0004
58/2	WI-38	9.1		0.02	0.00067
59/2a	WI-38	9.7			0.0011

a. 8×10^6 r.
b. This calculation based on inoculation of 0.5-ml amount of vaccine.

TABLE 3. EFFECT OF IRRADIATION ON POTENCY OF VENEZUELAN EQUINE ENCEPHALITIS VACCINES FOR GUINEA PIGS

Vaccine	Cell Culture Preparation	ED_{50}, ml, per Irradiation Dose 8×10^6 r	10×10^6 r	16×10^6 r	32×10^6 r
MR27[a]	MTCE	0.0016	0.0020	0.04	>0.04
58/2[b]	WI-38	0.00067	0.01		

a. Animals vaccinated with two inoculations of 0.2 ml.
b. Animals vaccinated with three inoculations of 0.2 ml.

C. RESISTANCE OF VACCINATED GUINEA PIGS TO CHALLENGE

Because guinea pigs immunized with irradiated VEE vaccines resisted challenge with $10^{5.4}$ $MICLD_{50}$ of virulent VEE virus, it was of interest to determine the upper limits of protection afforded by vaccination. A total of 105 animals were immunized with three inoculations of 0.25-ml amounts of undiluted vaccine (MTCE) exposed to 8×10^6 r. The animals were divided into groups of 15 each and challenged 21 days later with graded doses of VEE virus ranging from $10^{2.4}$ to $10^{8.4}$ $MICLD_{50}$. All vaccinated animals survived challenge. Serum samples were taken from two animals selected at random from each group immediately prior to challenge and 14 days postchallenge. Prechallenge sera contained high levels of SN (5.1 to 7.3) and moderate to high levels of HI antibody (160 to >10,240). Five of 12 guinea pigs challenged with doses of $10^{5.4}$ $MICLD_{50}$ or greater responded with a fourfold or greater increase in postchallenge HI titer.

D. ANTIBODY RESPONSE OF RABBITS TO IRRADIATED VEE VACCINE

Table 4 shows the HI and SN antibody titers obtained in the sera of two rabbits given a series of five injections of 0.5 ml of irradiated vaccine, lot MR27, on days 0, 3, 5, 9, and 11, followed by a booster dose of 0.5 ml at 15 months. Peak HI and SN titers were high and occurred 1 week after completion of the initial vaccination series. The HI titer in one rabbit was still at a significant level 10 months later, but was barely detectable in the other rabbit. Both rabbits exhibited lower but significant SN titers at that time. A single booster dose of vaccine stimulated a rapid and substantial increase in HI and SN antibodies at least equal to the original peak titer levels.

TABLE 4. HEMAGGLUTINATION-INHIBITING AND SERUM-NEUTRALIZING ANTIBODY RESPONSE IN RABBITS VACCINATED WITH IRRADIATION-INACTIVATED VENEZUELAN EQUINE ENCEPHALITIS VACCINE

Weeks Post-vaccination	Rabbit 1		Rabbit 2	
	Reciprocal of HI	Log_{10} SN	Reciprocal of HI	Log_{10} SN
1	5,120	8.4	2,560	7.3
2	640	8.4	640	8.4
3	320	ND[a/]	320	ND
40	80	3.6	10	3.2
60 (booster)	20	3.3	10	2.7
61	5,120	8.4	10,240	8.4
62	>20,480	8.4	5,120	8.4
63	10,240	8.4	5,120	8.4

a. ND = not done.

IV. DISCUSSION

VEE vaccines inactivated by gamma irradiation of virus suspensions prepared in tissue cultures exhibit high potencies. Irradiated vaccines stimulate the production of high levels of HI and SN antibodies when administered to animals in a series of two or more injections.

There appears to be little difference in the potencies of irradiated vaccines prepared from infected WI-38 monolayer or MTCE tissue cultures. However, the antigenicity of the WI-38 vaccine was more sensitive than that of the MTCE vaccine to the deleterious action of high doses (10×10^6 r) of radiation. This may be due to the presence of lower amounts of proteinaceous material in the WI-38 preparation. Polley[18] reported that influenza virus hemagglutinin of purified suspensions was destroyed more rapidly than infectivity, and that the addition of radioprotective agents such as histidine, ascorbic acid, or cysteine reversed this effect. Nagle's medium,[17] used for growth and maintenance of MTCE, contains greater amounts of radioprotective substances than the BME medium used for WI-38 cells.

It should be noted that five of 12 guinea pigs challenged with doses of $10^{5.4}$ $MICLD_{50}$ or greater responded with a significant rise in HI antibody titer. Increased antibody production could be attributed to active infection or to an anamnestic response to the challenge dose. The high SN antibody levels present at the time of challenge appear to support the latter concept.

An excellent immune response was obtained in rabbits. Although antibody levels decreased considerably 10 to 15 months after vaccination, significant levels of SN antibody were still present at that time, and a single booster dose rapidly restored circulating antibody to the original levels.

The data presented here indicate that inactivation by ionizing radiation may be an excellent method for the inactivation of virus in the preparation of virus vaccines. This conclusion is supported by the degree of protection afforded vaccinated guinea pigs against challenge with doses up to $10^{8.4}$ $MICLD_{50}$ of virus.

Highly potent inactivated VEE vaccines prepared by irradiation with gamma rays may be applicable to immunization of animals, such as equines and fowls, in an attempt to break the VEE virus - arthropod cycle of infection in epidemic and endemic areas. An irradiated vaccinia virus vaccine has been tested in humans previously immunized against smallpox with a live vaccine.[18] Administration of the irradiated (noninfectious) antigen stimulated an antibody response and produced a maculopapular or vesicular reaction of the delayed hypersensitivity type.

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13. ABSTRACT

The efficacy of formalin-inactivated Venezuelan equine encephalitis (VEE) vaccine has been reported to be low for man. Although a live VEE vaccine has been shown to be highly effective for the protection of laboratory workers, local and systemic reactions have occurred in approximately 20% of inoculated individuals. Therefore, studies were initiated in an attempt to produce an inactivated vaccine of high potency and low reactogenicity. Inactivated VEE vaccines were prepared by exposing virus suspensions to 8×10^6 or 10×10^6 r of gamma radiation. Irradiated VEE vaccines prepared from virus suspensions produced in Maitland-type chick embryo (MTCE) cell cultures and in monolayer cultures of human diploid strain WI-38 cells were highly immunogenic for mice and guinea pigs. Guinea pigs vaccinated with a series of three inoculations of vaccine (MTCE) survived challenge with at least $10^{8.4}$ $MICLD_{50}$ of VEE virus. Irradiated vaccines induced high levels of serum-neutralizing and hemagglutinin-inhibiting antibodies in guinea pigs and rabbits. These findings suggest that ionizing radiation may be effective in the preparation of an inactivated VEE vaccine.

14. Key Words

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